

Inferring population connectivity across the range of distribution of the stiletto shrimp *Artemesia longinaris* Spence Bate, 1888 (Decapoda, Penaeidae) from DNA barcoding: implications for fishery management

Abner Carvalho-Batista¹, Mariana Negri², Leonardo G. Pileggi²,
Antonio L. Castilho³, Rogério C. Costa¹, Fernando L. Mantelatto²

1 Laboratory of Biology of Marine and Fresh Water Shrimps, Faculty of Science, Department of Biological Sciences, São Paulo State University (UNESP), Bauru, São Paulo, Brazil **2** Laboratory of Bioecology and Crustacean Systematics, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto (FFCLRP), University of São Paulo (USP), Postgraduate Program in Comparative Biology, Ribeirão Preto, São Paulo, Brazil **3** São Paulo State University (UNESP), Biosciences Institute of Botucatu, Zoology Department, Botucatu, Brazil

Corresponding author: Fernando L. Mantelatto (flmantel@usp.br)

Academic editor: I.S. Wehrtmann | Received 5 November 2013 | Accepted 21 March 2014 | Published 25 November 2014

<http://zoobank.org/172EC335-1475-40A9-AD46-D5D45ABD8F9E>

Citation: Carvalho-Batista A, Negri M, Pileggi LG, Castilho AL, Costa RC, Mantelatto FL (2014) Inferring population connectivity across the range of distribution of the stiletto shrimp *Artemesia longinaris* Spence Bate, 1888 (Decapoda, Penaeidae) from DNA barcoding: implications for fishery management. In: Wehrtmann IS, Bauer RT (Eds) Proceedings of the Summer Meeting of the Crustacean Society and the Latin American Association of Carcinology, Costa Rica, July 2013. ZooKeys 457: 271–288. doi: 10.3897/zookeys.457.6569

Abstract

Artemesia longinaris is a marine shrimp endemic to the southwestern Atlantic and distributed from Atafona, Rio de Janeiro (Brazil) to Rawson, Chubut (Argentina). In recent years, this species has become an important target of the commercial fishery as a consequence of the decline in the fishery of more traditional and profitable marine shrimps. In addition, phenotypic variations have been documented in populations along its distribution. Therefore, investigations on the genetics of the fishing stocks are necessary for the development of sustainable management strategies and for understanding the possible sources of these variations. The mitochondrial gene Cytochrome Oxidase I (COI) was used to search for evidence of genetic structure among the populations of *A. longinaris* and to analyze the phylogenetic relationships among them. A total of 60 specimens were collected from seven different localities, covering its geographical range. The final alignment showed 53 haplotypes (48 individuals and 5 shared), with no biogeographical pattern. The low genetic divergence found, with a non-significant F_{ST} value, also suggests the absence of population structure for this

gene. These findings indicate a continuous gene flow among the populations analyzed, suggesting that the phenotypic variation is a consequence of different environmental conditions among the localities.

Keywords

Cytochrome Oxidase I, gene flow, Penaeoidea, phenotypic plasticity

Introduction

Artemesia longinaris Spence Bate, popularly known as Argentine stiletto shrimp, plays an important role in the marine trophic chain of the southwestern Atlantic, as food for different species of fish and cephalopods (Capitoli et al. 1994). In recent years, however, this species has become a common target of both artisanal and industrial fisheries. The former occurs along its entire distribution and the latter is mainly concentrated in southern Brazil and Argentina (D’Incao et al. 2002). The increase in the fishery of *A. longinaris* is a consequence of a decline in the stocks of more traditional and profitable marine shrimps, such as the pink shrimp *Farfantepenaeus brasiliensis* (Latreille) and *F. paulensis* (Pérez-Farfante), the white shrimp *Litopenaeus schmitti* (Burkenroad) and the seabob shrimp *Xiphopenaeus kroyeri* (Heller) (D’Incao et al. 2002, Costa et al. 2004, Carvalho-Batista et al. 2011).

In the last decades, catches in the states of south and southeast Brazil have reached thousands of tons (D’Incao et al. 2002). Furthermore, in spite of the increase in its exploitation in recent years, there is no specific management plan for *A. longinaris* in Brazil. The offseason in south and southeast coast of this country for this species and other commercial shrimps is based on the period of juvenile recruitment of *Farfantepenaeus* species, without taking account the possibility of the existence of more than one stock for these species (Franco et al. 2009).

Artemesia longinaris has a distribution restricted to the southwestern Atlantic, from Atafona (Rio de Janeiro, Brazil, 21°37'S) to Rawson (Chubut, Argentina, 43°18'S) (D’Incao 1999). Although its distribution is limited to the Argentinean biogeographical province, much of its extent (23° to 35°S) is considered a transitional region because of current mixing; this process leads to the formation of water masses with tropical and subantarctic characteristics (Boschi 2000). In addition, the northern boundary of its distribution is located in the region of Cabo Frio (Rio de Janeiro, Brazil), where there is a strong influence of upwelling events, driven by the winds and coastal topography (Acha et al. 2004).

Consequently, environmental conditions differ considerably throughout the range of *A. longinaris*. For example, in the Ubatuba region (São Paulo, Brazil) the temperature (16–30 °C) and salinity (28–38) vary widely because of the intrusion of different water masses (Fransozo et al. 2004, Costa et al. 2005); whereas near Cabo Frio (Rio de Janeiro, Brazil) the water temperature is about 20 °C and the salinity is high (>37) during most of the year (Sancinetti 2011); and on Mar del Plata coast (Buenos Aires, Argentina) the temperature varies seasonally, from 6 to 17 °C, and the salinity is slightly greater than 30 (Petriella and Bridi 1992, Guerrero et al. 1997, Acha et al. 2003).

In addition, phenotypic variations among *A. longinaris* populations have been noted. The body size and the mean size at sexual maturity ($CL_{50\%}$) increase with the lati-

tude, from Ubatuba (São Paulo, Brazil) to Mar del Plata (Buenos Aires, Argentina), but decrease with latitude from the Farol de São Tome (Rio de Janeiro, Brazil) to Ubatuba (Boschi 1969a, Ruffino and Castello 1992, Castilho et al. 2007b, Semensato and Di Benedetto 2008, Costa et al. 2010). Differences in certain morphometric relationships have also been detected (Dumont and D’Incao 2010), as well as in the reproductive period, which tends to be continuous in lower latitudes and seasonal in higher latitudes (Christiansen and Scelzo 1971, Petriella and Bridi 1992, Castilho et al. 2007a).

In view of these environmental variations, Nascimento (1983) proposed that the populations off southern Brazil and northern Argentina are likely separated, based on the differences in their environmental preferences. However, an analysis of enzyme polymorphisms provided no support for this proposition (Weber et al. 1993). Further studies to investigate the possibility of genetic structure and covering the entire distribution of *A. longinaris* were still lacking.

Knowledge of the genetic structure of populations is important for the development and success of strategies for sustainable long-term management of fishery resources (Hillis et al. 1996). Mitochondrial DNA has been an important tool for these investigations, for terrestrial as well as aquatic organisms (Avise 1994). Among the mitochondrial molecular markers, the Cytochrome Oxidase I (COI) gene has been successfully employed to detect population structures in many species of Decapoda (Schubart and Huber 2006, Aoki et al. 2012, De Croos and Pálsoon 2012, Terossi and Mantelatto 2012). This property, together with other characteristics, has resulted in the choice of this gene as the standard marker for animal identification in the DNA barcoding technique (Hebert et al. 2003).

This study had the following aims: to evaluate the hypothesis of genetic structure among the populations of *A. longinaris*; investigate their phylogenetic relationships; and detect, if possible, evidences of speciation. To achieve these purposes, we used a partial sequence of the mitochondrial COI gene as the molecular marker. The population concept adopted was proposed by Roughgarden et al. (1989) and Krebs (1994). According to them, a population is a group of organisms of the same species that occupy the same place at a certain time. Our findings provide an appropriate theoretical basis for the development of management strategies for this fishery resource, as well as help to understand the origin of the phenotypic differences among populations of this species.

Methods

Sample collection

The specimens were obtained, at scientific cruises, from seven localities in the southwestern Atlantic (Table 1 and Fig. 1). The specimens were identified based on Costa et al. (2003), and were immediately preserved in 80% ethanol and deposited in the Crustacean Collection of the Department of Biology (CCDB), Faculty of Philosophy, Sciences and Letters at Ribeirão Preto (FFCLRP), University of São Paulo (USP) (Table 1).

Table 1. List of specimens used for molecular analysis with respective site of collection, catalogue numbers, and GenBank accession numbers for *Artemesia longinaris*. The letters CCDB preceding the catalogue numbers represent the Crustacean Collection of the Department of Biology, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, University of São Paulo.

Locality	Catalogue numbers	GenBank Accession Numbers
Macaé-Rio de Janeiro, Brazil (22°23'44"S; 41°44'57"W)	CCDB 3782	KF572060–KF572069
Ubatuba-São Paulo, Brazil (23°27'24"S; 45°01'20"W)	CCDB 3806, 3429	KF572070–KF572082
Santos-São Paulo, Brazil (24°03'59"S; 46°16'57"W)	CCDB 4008	KF572083–KF572084
Cananéia-São Paulo, Brazil (25°08'15"S; 47°50'40"W)	CCDB 3655	KF572085–KF572089
São Francisco do Sul-Santa Catarina, Brazil (26°05'52"S; 48°33'82"W)	CCDB 3851	KF572090–KF572098
Rio Grande-Rio Grande do Sul, Brazil (32°10'23"S; 52°06'10"W)	CCDB 3928	KF572099–KF572108
Mar del Plata-Buenos Aires, Argentina (37°58'57"S; 57°32'15"W)	CCDB 869, 4150	KF572109–KF572119

DNA extraction, PCR amplification, purification and sequencing

The protocols for DNA extraction, amplification and sequencing followed Mantelatto et al. (2009) and Pileggi and Mantelatto (2010).

An ~700-bp region of a partial sequence of the mitochondrial COI gene was amplified by the polymerase chain reaction (PCR) using the pair of primers: HCO1 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and LCO1 (5'-GGT-CAACAAATCATAAAGATATTGG-3') (Folmer et al. 1994). The PCR reaction was performed in an Applied Biosystems Veriti® 96-well thermocycler, using the following thermal cycle: initial denaturing for 2 min at 94 °C followed by 35 denaturing cycles at 94 °C for 30 s, primer annealing at 50–58 °C for 30 s and extension at 72 °C for 1 min, and a final extension for 5 min at 72 °C. The PCR products were purified using the SureClean Plus® purification kit (Bioline) and were sequenced with the Big Dye® Terminator Cycle Sequencing kit in an ABI 3100 Genetic Analyzer® (Applied Biosystems Life Technologies). All sequences were confirmed by sequencing both strands.

Data analysis

The editing and construction of a consensus sequence for the two strands were conducted using the computational program BIOEDIT 7.3.1.0 (Hall 1999). Sequences were aligned using the program CLUSTAL W (Thompson et al. 1994), with interface to BIOEDIT (Hall 1999) using default parameters. The computational program MEGA 5.0 (Tamura et al. 2011) was used to estimate the average nucleotide composition and

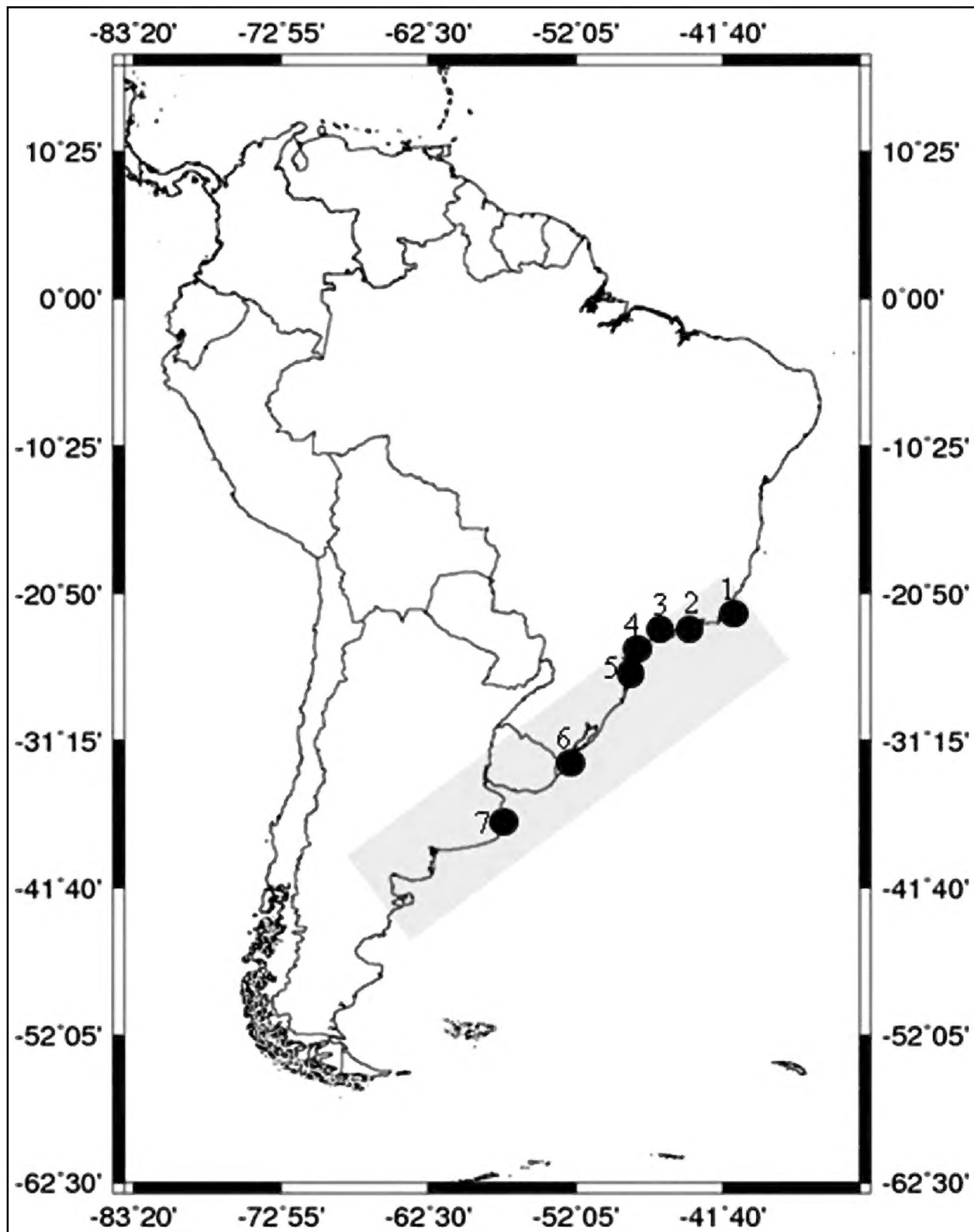


Figure 1. Southwest Atlantic collection sites. Map showing the localities of the specimens of *Artemesia longinaris* analyzed: **1** Macaé, Brazil **2** Ubatuba, Brazil **3** Santos, Brazil **4** Cananéia, Brazil **5** São Francisco do Sul, Brazil **6** Rio Grande, Brazil **7** Mar del Plata, Argentina. The gray band indicates the complete geographical distribution of *Artemesia longinaris*.

genetic distances, and to construct a Neighbor-Joining dendrogram, both based on the Kimura 2-parameter substitution model (Kimura 1980). The phylogram using the Maximum Likelihood criterion was constructed in the program RAxML-HPC2 on X-SEDE (Stamatakis 2006) through the online version of the Cyber Infrastructure for Phylogenetic Research (CIPRES) website (Stamatakis et al. 2008, Miller et al. 2010). The default parameters of RAxML were used to perform the analysis for the GTR model. To measure the consistency of the topology, we selected the option to automati-

cally determine the number of bootstraps to be run in RAxML. Consequently, 1000 bootstrap pseudo-replicates were run, and only the values >50% were reported.

For both the genetic distance and phylogenetic analyses, sequences of three other penaeid species were included in the alignment as an outgroup: *F. brasiliensis*, *F. paulensis* (GenBank accession numbers KF783861–KF783862) and *Rimapenaeus constrictus* (Stimpson) (GenBank accession number KF783863). We also attempted to use a sequence of the same portion of the COI gene of *A. longinaris* available in GenBank (accession number EU400383.1) (Dumont et al. 2009). However, it was not possible to obtain alignments without gaps when this sequence was included. This observation, allied to the fact that its translation to an amino-acid sequence showed the presence of stop codons, indicates that this sequence must be reviewed. The presence of stop codons in the middle of an encoding gene suggests the possibility of the amplification and sequencing of a pseudogene (Buhay 2009).

The haplotype number was calculated in the program DNASP 4.10.9 (Rozas and Rozas 1999). The haplotype network was constructed by the Median-Joining method in NETWORK software (Bandelt et al. 1999), with data preparation in DNASP. The haplotype and nucleotide diversities were calculated for each locality using ARLEQUIN Version 3.1 (Excoffier et al. 2005). The genetic variation was analyzed with a analysis of molecular variance (AMOVA) (Excoffier et al. 1992), and was computed in ARLEQUIN Version 3.1 (Excoffier et al. 2005).

Results

A total of 60 sequences of the COI gene from individuals sampled in the seven localities was obtained. The final multiple sequence alignment included 645 base pairs. The number of variable sites was 66 (10.23%), 8 (12.12%) in the first codon position and 58 (87.88%) in the third position, and 30 of the variable sites were phylogenetically informative. Adding three species as the outgroup, the number of variable sites was 143 (28.49%), 72 of which were phylogenetically informative. The average nucleotide composition for *A. longinaris* was 28.41% (A), 30.99% (T), 19.47% (G), and 21.12% (C).

The intraspecific genetic distance of *A. longinaris* ranged from 0 to 2.7%, and the average distance was $1.1 \pm 0.2\%$. The interspecific genetic distance, including the outgroup, ranged from 21.3 to 27.1%. Average distance among individuals in each population ranged from $0.81 \pm 0.25\%$ at Cananéia to $1.42 \pm 0.24\%$ at Macaé (Table 2). Among localities, distances ranged from $0.8 \pm 0.2\%$ between Santos and Cananéia to $1.4 \pm 0.2\%$ between Macaé and São Francisco do Sul (Table 3).

Both the Neighbor-Joining and Maximum Likelihood analysis indicated no structure by localities (Figs 2 and 3).

Based on the 60 sequences, 53 haplotypes were identified. Of these, 48 represented single individuals. The locality of Santos was not included in the analysis of haplotype, nucleotide diversity and molecular variance (Tables 4 and 5), since only two sequenc-

Table 2. Average distance (%) among *Artemesia longinaris* individuals \pm standard deviation in each locality.

Locality	Average distance (%)	Standard deviation (\pm)
Macaé	1.42	0.24
Ubatuba	1.07	0.19
Santos	1.25	0.43
Cananéia	0.81	0.25
São Francisco do Sul	1.37	0.26
Rio Grande	1.08	0.19
Mar del Plata	0.88	0.21

Table 3. *Artemesia longinaris*: average distance (%) among localities (numbers on bottom) \pm standard deviation (values on top).

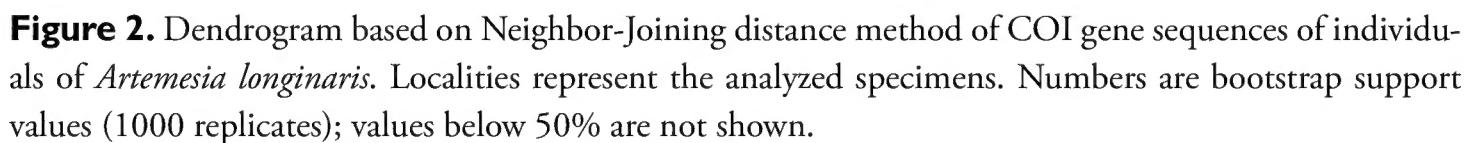
Locality	1	2	3	4	5	6	7
1 Macaé		0.19	0.25	0.21	0.22	0.19	0.19
2 Ubatuba	1.21		0.23	0.18	0.19	0.18	0.17
3 Santos	1.30	1.08		0.23	0.23	0.23	0.23
4 Cananéia	1.17	0.95	0.78		0.20	0.18	0.19
5 São Francisco do Sul	1.37	1.21	1.13	1.02		0.19	0.20
6 Rio Grande	1.20	1.04	1.13	0.96	1.20		0.17
7 Mar del Plata	1.16	0.96	0.97	0.83	1.11	0.95	

Table 4. Number of *Artemesia longinaris* individuals sampled, number of haplotypes, D.H. = haplotype diversity, and D.N. \pm D.P. = nucleotide diversity \pm standard deviation for each locality.

Locality	Number of samples	Number of haplotypes	D. H.	D. N. \pm D. P.
Macaé	10	10	0.10	$1.38 \times 10^{-3} \pm 0.79 \times 10^{-3}$
Ubatuba	13	13	0.08	$1.05 \times 10^{-3} \pm 0.4 \times 10^{-3}$
Santos	2	2		
Cananéia	5	5	0.20	$0.80 \times 10^{-3} \pm 0.5 \times 10^{-3}$
São Francisco do Sul	9	9	0.11	$1.34 \times 10^{-3} \pm 0.8 \times 10^{-3}$
Rio Grande	10	9	0.12	$1.05 \times 10^{-3} \pm 0.6 \times 10^{-3}$
Mar del Plata	11	11	0.91	$0.87 \times 10^{-3} \pm 0.5 \times 10^{-3}$

es were obtained from this site. The caught of *Artemesia longinaris* in this locality is difficult, occurring only in some occasions with low temperatures and often in low abundances (Carvalho-Batista et al. 2011). The haplotype network did not reveal any genetic structure among groups (Fig. 4). Five haplotypes were shared, and the most frequent one was observed in four specimens from three localities (Fig. 4).

The analysis of molecular variance (AMOVA) did not detect structure among the localities, and the observed variation occurred predominantly within the localities. The F_{ST} indices were not significant ($p > 0.05$) (Table 5).



The intraspecific genetic distance for *A. longinaris* (0–2.7%) is much lower than the interspecific distance between *A. longinaris* and the out-group species (21.3–27.1%). This result not only confirms *A. longinaris* as a single taxon throughout its distribution, but also supports the utilization of this methodology in the identification of penaeid shrimps from the Brazilian coast. The difference between the intra and interspecific genetic variation of the barcode region of the COI gene is termed the “barcode gap” (Hebert et al. 2004). It is an efficient method for differentiating species through the DNA Barcoding technique (Hebert et al. 2004, Waugh 2007, Frézal and Leblois 2008, Ward 2009). The genetic divergence values are consistent with other studies involving

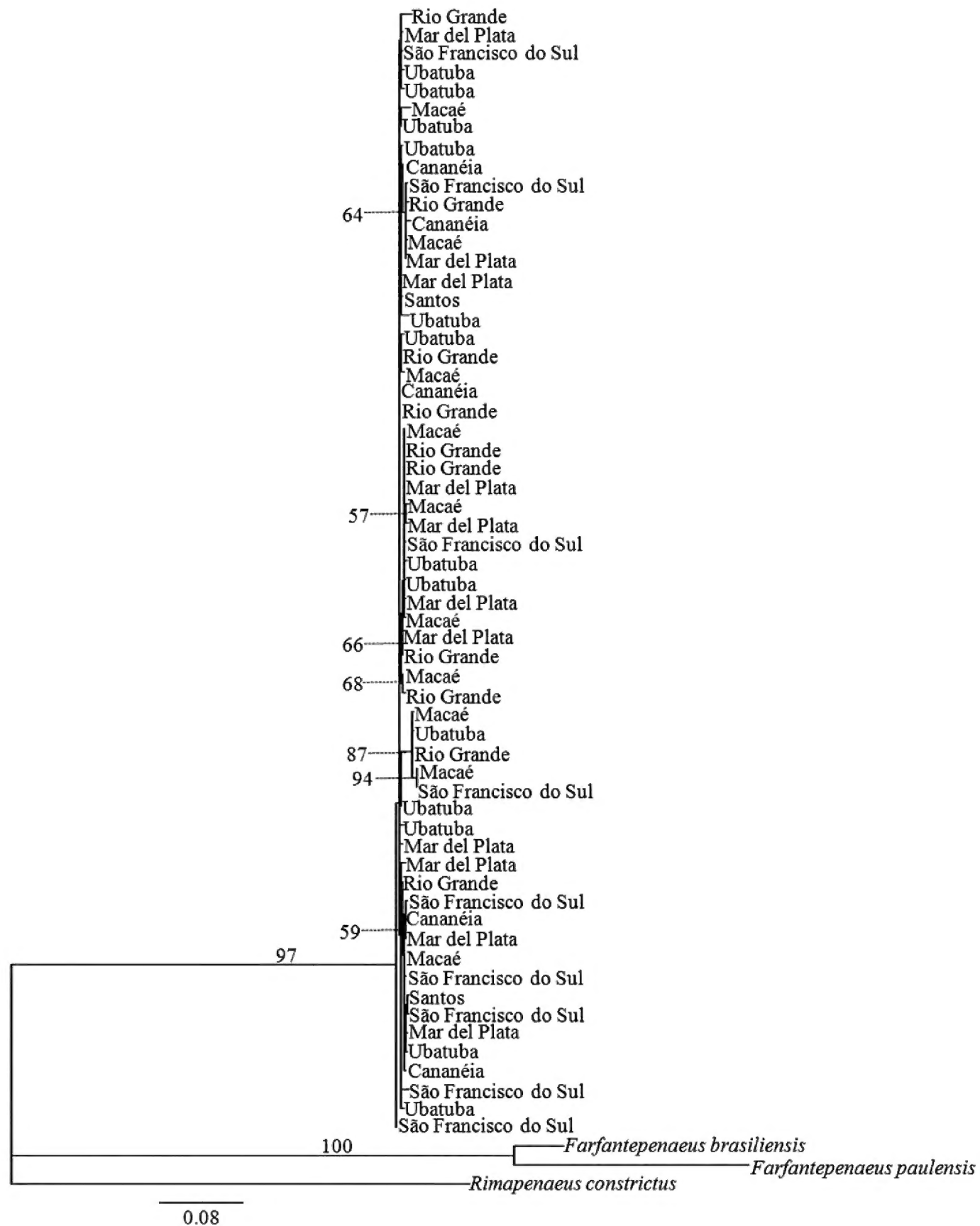


Figure 3. Phylogram for individuals of *Artemesia longinaris* inferred from Maximum Likelihood analysis of COI gene sequences. Localities represent analyzed specimens. Numbers are bootstrap support values (1000 replicates); values below 50% are not shown.

Table 5. Analysis of molecular variance (AMOVA) performed with specimens of *Artemesia longinaris* obtained from seven localities. *Significant values, $P < 0.05$.

Structure	Variation Source	%	Fixation index	P
Absent	Among localities	-1.80	FST: -0.02	0.95
	Within localities	101.80		

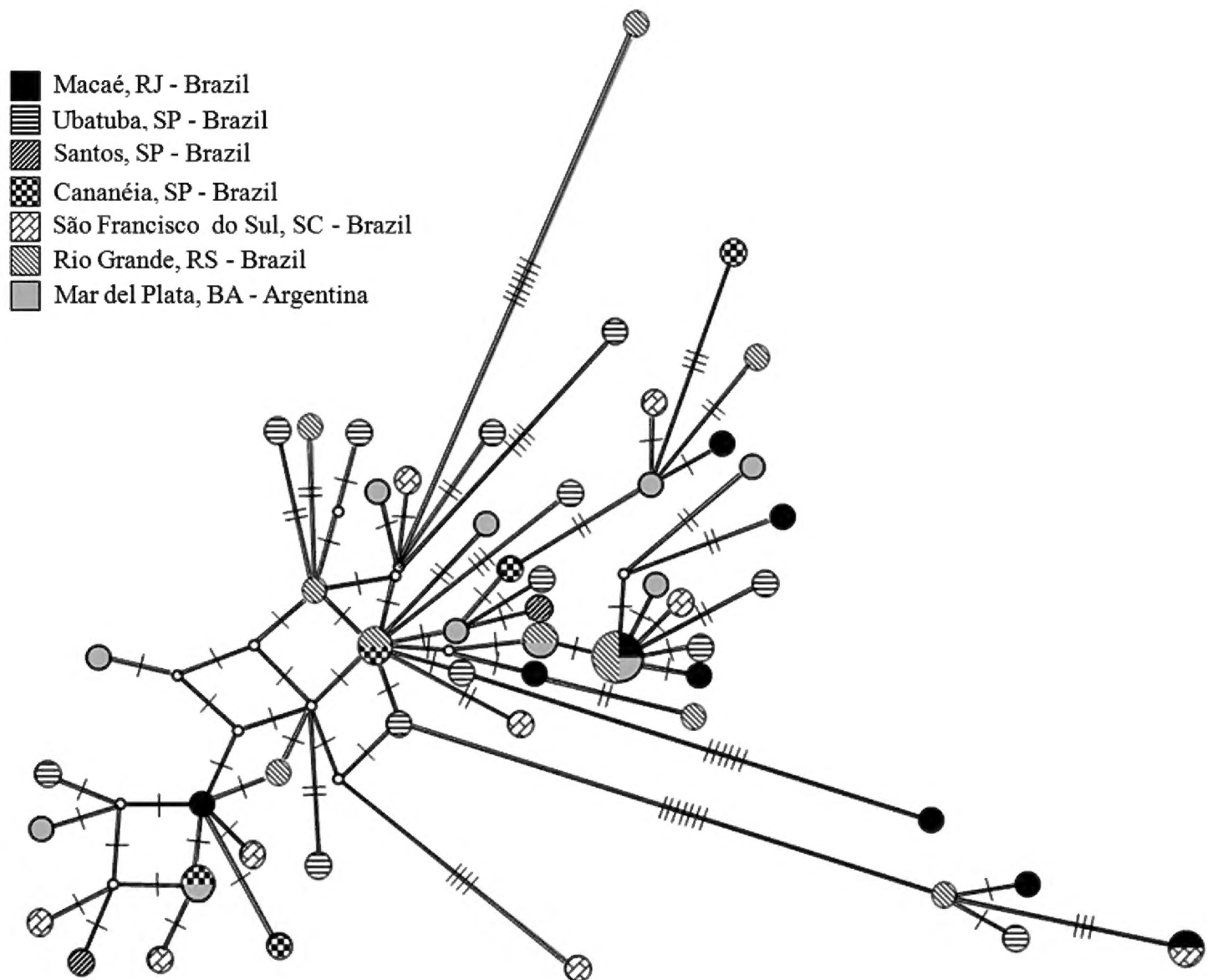


Figure 4. Haplotype network of *Artemesia longinaris* according to Median-Joining analysis. Each circle represent one haplotype found in the localities (53 haplotypes in 60 specimens). The size of the circle of each haplotype is proportional to its frequency in the sample. Each small dash represents a mutational step.

the family Penaeidae, with intraspecific values lower than 3.5% and interspecific values generally higher than 10% (in some cases exceeding 20%) (Gao et al. 2003, Quan et al. 2004, Keskin and Atar 2013).

Our analyses showed genetic homogeneity among the populations of *A. longinaris* along its entire geographical distribution. The F_{ST} value obtained reflects this absence of geographical genetic structure. In species with high genetic variation and few shared haplotypes, negative F_{ST} values are probably associated with the imprecision of the algorithms used in this type of analysis, and can be interpreted as zero (Winkelmann et al. 2013).

Despite the absence of significant genetic variability at the intraspecific level described here, phenotypic variability was previously observed among the populations of *A. longinaris* (see introduction). The determination of an individual phenotype is a consequence of the interaction between genotype and environment (Templeton 2006). Thus, the same genotype may be associated with different phenotypes under different environmental conditions (Miner et al. 2005, Vogt et al. 2008, Sotka 2012).

Recent studies with other decapods, with sampling at several points of the South American coast, found similar results on genetic homogeneity (Laurenzano et al. 2012, Terossi and Mantelatto 2012, Rossi and Mantelatto 2013, Wieman et al. 2013, Laurenzano et al. 2013). These authors indicated the high capacity of planktonic larval dispersal as the main factor responsible for this homogeneity over their distributions, making it impossible to establish a population structure over this broad geographical range (Gopurenko and Hughes 2002).

We can conjecture that similar larval dispersal occurs with *A. longinarius*, in which its larval development lasts 24 to 32 days, according to the temperature (Boschi and Scelzo 1977). This period is sufficient for the larvae to be passively transported for hundreds of kilometers by the currents (Palumbi 2003). The ability of larvae to travel for long distances was demonstrated for other penaeid shrimps. For example, larvae of *Pleoticus muelleri* (Spence Bate), on the Argentine coast, are able to travel for distances between 120 and 300 nautical miles (about 220 and 550 km, respectively), transported by the coastal currents (Boschi 1989).

It is thought that the dynamics of water masses in the region provides ideal conditions for larval drift of *A. longinarius* through the southwestern Atlantic. Coastal Water (CW), for instance, is a water mass that cover the geographical range of this study (Campos et al. 2000), and can flow towards north or south depending on the wind conditions and season showing different properties of temperature and salinity, depending the region and the influence of other water masses (Piola et al. 2005, Calado et al. 2006, Castro-Filho et al. 2008) allowing larval dispersal to different areas.

According to Fransozo et al. (2004), Costa et al. (2005) and Carvalho-Batista et al. (2011), the occurrence of adults of *A. longinarius* in São Paulo State is associated with the temperature decrease to 17–21 °C. During the spring (October to December) in Ubatuba, the number of animals in the larger size classes increased. It was associated with the coming of migrants into the population (Castilho et al. 2007a). Thus, the gene flow of *A. longinarius* is not limited to larval drift, but also is a consequence of juvenile and adult migration. Penaeid migration over long distances was also evidenced by Ruello (1975), who recaptured a female of *Melicertus plebejus* (Hess), on the Australian coast, 930 km from the site where the specimen was marked.

Our results, encompassing samples from its entire distribution, support the hypothesis that *A. longinarius* migrates over long distances, and is able to establish populations in different areas when conditions are appropriate. It is therefore possible to consider *A. longinarius* as a metapopulation, which fits the model of source and sink proposed by Pulliam (1988). The populations (or subpopulations) that are continuously more stable and in high density throughout the year, such as those from southern Brazil to Argentina and from Macaé (Boschi 1969ab, Nascimento 1983, Sancinetti 2011), are probably sources of new individuals for the less-stable populations, the sinks, such as the populations (or subpopulations) from São Paulo State.

Thus, these localities, where the populations are considered sources, would be strategic for the implementation of management measures such as the creation of protected areas or offseason periods, in order to maintain the fisheries in these areas and also in all

range of its distribution. The role of marine protected areas in enhance fisheries in adjacent regions depend if they act as sources or as sinks (King 1995). Even connected one to each other, each subpopulation has its own dynamic (Begon et al. 2006), so the conservation policies must take into account the particular characteristics of each locality.

Studies investigating the larval dispersal and the migration of juveniles and adults of this species must be conducted in order to verify whether the model described by Pulliam (1988) is applicable or not. Apart from this, with the intent of providing a better quantification of the degree of exchange among the populations, as well as to evaluate the possibility of recent divergence among them, which is not detectable by the marker used here, additional molecular investigations using different genes are encouraged.

Conclusion

Our results confirm that the DNA barcoding technique is an efficient tool for the identification of penaeid shrimps from the Brazilian coast. In addition to the validation of *A. longinaris* as a single taxon, with no genetic differentiation among the populations through its entire geographical distribution, we showed the importance of the effect of the environmental conditions specific to each locality in the expression of the phenotypic characteristics of the individuals in a population.

The genetic homogeneity is maintained by the larval dispersal and high migratory capacity, which assure gene flow among populations. These characteristics make it possible for individuals to be transported by water masses and currents of the southwestern Atlantic.

In addition, this study also indicate the importance of populations of south Brazil and Macaé as sources, to provide individuals to other areas. Thus these populations should be considered essential in developing management strategies for the species.

Acknowledgements

This paper is part of the multidisciplinary research project Temático BIOTA – FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), which aims to produce a fine-scale assessment of the marine decapod biodiversity of the State of São Paulo. Financial support for this project was provided by research grants from FAPESP (Temático Biota 2010/50188-8; Coleções Científicas 2009/54931-0) and CNPq (Proc. 301359/2007-5; 473050/2007-2; 504322/2012-5) to FLM. ACB thanks CNPq for a MSc scholarship (#130655/2011-2), MN thanks FAPESP for an ongoing PhD scholarship (2012/06300-3), LGP thanks CAPES for an ongoing Postdoctoral scholarship (02630/2009-5), and FLM and RCC acknowledge CNPq for research grants (PQ 302748/2010-5 and 304784/2011-7, respectively). We are extremely grateful to several colleagues and friends (Emiliano O'Campo, Laura Lopez-Greco, Mariana Terossi and Rafael Robles) for their help in collections, for making available some essential fresh specimens, for lending material from collections used in our research, for critical

discussions during the preparation of this manuscript, and for help in the sequencing step. Special thanks to all members of LBSC for their assistance during the development of this study and to Instituto Oceanográfico (IO/USP) for logistical support during field work. We also thank to Dr. Janet W. Reid (JWR Associates) for providing the English review service and anonymous reviewers for all comments and suggestions. The collections of species conducted in this study complied with current applicable state and federal laws of Brazil (DIFAP/IBAMA/126/05; permanent license to FLM for collection of Zoological Material No. 11777-1 MMA/IBAMA/SISBIO).

References

- Acha EM, Mianzan HW, Guerrero RA, Favero M, Brava J (2004) Marine fronts at the continental shelves of austral South America: physical and ecological processes. *Journal of Marine Systems* 44: 83–105. doi: 10.1016/j.jmarsys.2003.09.005
- Acha EM, Mianzan HW, Iribarne O, Gagliardini DA, Lasta C, Daleo P (2003) The role of Rio de La Plata bottom salinity front in accumulating debris. *Marine Pollution Bulletin* 46: 197–202. doi: 10.1016/S0025-326X(02)00356-9
- Aoki M, Nakagawa Y, Kawamoto M, Wada K (2012) Population divergence of the sentinel crab *Macrophthalmus banzai* is consistent with regional breeding season differences. *Zoological Science* 29: 821–826. doi: 10.2108/zsj.29.821
- Avise JC (1994) *Molecular markers, Natural History and Evolution*. Chapman & Hall, New York, 511 pp. doi: 10.1007/978-1-4615-2381-9
- Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16: 37–48. doi: 10.1093/oxfordjournals.molbev.a026036, <http://mbe.oxfordjournals.org/content/16/1/37>
- Begon M, Townsend CR, Harper JL (2006) *Ecology. From Individuals to Ecosystems*. Blackwell Publishing, Oxford, 738 pp.
- Boschi EE (1969a) Crecimiento, migración y ecología del camarón comercial *Artemesia longinaris* Bate, 1888, de Mar del Plata. *FAO Fisheries Report* 57: 833–846.
- Boschi EE (1969b) Estudio biológico pesquero del camarón *Artemesia longinaris* Bate, 1888 de Mar del Plata. *Boletín del Instituto de Biología Marinha, Mar del Plata* 18: 1–47.
- Boschi EE (1989) Biología pesquera del langostino del litoral patagónico de Argentina (*Pleoticus muelleri*). *Contribuciones del Instituto Nacional de Investigación y Desarrollo Pesquero* 646: 5–71.
- Boschi EE (2000) Species of decapods crustaceans and their distribution in the American marine zoogeographic provinces. *Revista de Investigación y Desarrollo Pesquero* 13: 1–64. <http://www.oceandocs.org/handle/1834/2606>
- Boschi EE, Scelzo MA (1977) Desarrollo larval y cultivo del camarón comercial de Argentina *Artemesia longinaris* Bate (Crustacea, Decapoda, Penaeidae). *FAO Informes de Pesca* 159: 287–327.
- Buhay JE (2009) “COI-like” sequences are becoming problematic in molecular systematic and DNA barcoding studies. *Journal of Crustacean Biology* 29: 96–110. doi: 10.1651/08-3020.1

- Calado L, Gangopadhyay A, Silveira ICA (2006) A parametric model for the Brazil Current meanders and eddies off Southeastern Brazil. *Journal of Geophysical Research Letters* 33: L12602. doi: 10.1029/2006GL026092
- Campos EJD, Velhote D, Silveira ICA (2000) Shelf break upwelling driven by Brazil Current cyclonic meanders. *Geophysical Research Letters* 27: 751–754. doi: 10.1029/1999GL010502
- Capitoli RR, Bager A, Ruffino ML (1994) Contribuição ao conhecimento das relações tróficas bentônico-demersais nos fundos de pesca do camarão *Artemesia longinaris* Bate, 1888 na região da barra da Lagoa dos Patos, RS, Brasil. *Nauplius* 2: 53–74. <http://www.crustacea.org.br/artigos/>
- Carvalho-Batista A, Simões SM, Lopes M, Costa RC (2011) Ecological distribution of the shrimp *Pleoticus muelleri* (Bate, 1888) and *Artemesia longinaris* Bate, 1888 (Decapoda: Penaeoidea) in the Southeastern Brazilian littoral. *Nauplius* 19: 135–143. <http://www.crustacea.org.br/artigos/>
- Castilho AL, Fransozo A, Costa RC, Boschi EE (2007a) Reproductive biology of the shrimp *Artemesia longinaris* Bate, 1888 (Crustacea, Decapoda, Penaeidae) in the northern coast of São Paulo State, Brazil. *Revista de Biología Tropical* 55: 39–48. <http://www.redalyc.org/pdf/449/44909908.pdf>
- Castilho AL, Gavio MA, Costa RC, Boschi EE, Bauer RT, Fransozo A (2007b) Latitudinal variation in population structure and reproductive pattern of the endemic South American shrimp *Artemesia longinaris* (Decapoda: Penaeoidea). *Journal of Crustacean Biology* 27: 548–552. doi: 10.1651/S-2788.1, <http://decapoda.nhm.org/pdfs/31567/31567.pdf>
- Castro-Filho BM, Miranda LB, Silva LS, Fontes RFC, Pereira AF, Coelho AL (2008) Processos Físicos: Hidrografia, Circulação e Transporte. In: Pires-Vanin AMS (Eds) *Oceanografia de um Ecossistema Subtropical: Plataforma de São Sebastião, SP*. Editora da Universidade de São Paulo, São Paulo, 59–122.
- Christiansen HE, Scelzo MA (1971) Ciclo de maduración sexual y observaciones sobre la morfología del aparato genital del camarón *Artemesia longinaris* Bate. *Carpas* 16: 1–22.
- Costa RC, Fransozo A, Pinheiro MAP (2004) Ecologic distribution of the shrimp *Pleoticus muelleri* (Bate, 1888) (Decapoda: Penaeoidea) of Southeastern Brazil. *Hydrobiologia* 529: 195–203. doi: 10.1007/s10750-004-6410-x
- Costa RC, Fransozo A, Castilho AL, Freire FAM (2005) Annual, seasonal and spatial variation of abundance of the shrimp *Artemesia longinaris* (Decapoda: Penaeoidea) in south-eastern Brazil. *Journal of the Marine Biological Association of the United Kingdom* 85: 107–112. doi: 10.1017/S0025315405010908h
- Costa RC, Fransozo A, Melo GAS, Freire FAM (2003) An illustrated key for Dendrobranchiata shrimps from the northern coast of São Paulo state, Brazil. *Biota Neotropica* 3:1–12. doi: 10.1590/S1676-06032003000100011, <http://www.biotaneotropica.org.br/v3n1/pt/full-paper?bn01503012003+en>
- Costa RC, Branco JO, Machado IF, Campos BR, Avila MG (2010) Population biology of shrimp *Artemesia longinaris* (Crustacea: Decapoda: Penaeidae) from the southern coast of Brazil. *Journal of the Marine Biological Association of the United Kingdom* 90: 663–669. doi: 10.1017/S002531540999124X
- De Cross MDST, Pálsoon S (2012) Population biology and genetic diversity of two adjacent shrimp (*Parapenaeopsis coromandelica*) populations exploited under different fishing pres-

- tures in the coastal waters of Sri Lanka. *Journal of the Marine Biological Association of the United Kingdom* 92: 819–829. doi: 10.1017/S0025315411000828
- D’Incao F (1999) Subordem Dendrobranchiata (camarões marinhos). In: Buckup L, Bond-Buckup G (Orgs) *Os Crustáceos do Rio Grande do Sul*. Editora da Universidade, UFRGS, Porto Alegre, 271–299.
- D’Incao F, Valentini H, Rodrigues LF (2002) Avaliação da pesca de camarões nas regiões Sudeste e Sul do Brasil. 1965-1999. *Atlântica* 24: 49–62.
- Dumont LFC, D’Incao F (2010) Biometric relationships of the Argentinean prawn *Artemesia longinaris* (Decapoda: Penaeidae) in the south-western Atlantic. *Journal of the Marine Biological Association of the United Kingdom* 90: 1385–1393. doi: 10.1017/S0025315409990415
- Dumont LF, Hwang G, Maclean N (2009) The mtDNA control region of the barba-ruça shrimp *Artemesia longinaris* (Decapoda: Penaeidae) and its potential use as a marker for population analysis. *Atlântica* 31: 199–207. doi: 10.5088/atl.2009.31.2.199
- Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47–50. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2658868/>
- Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491. <http://www.genetics.org/content/131/2/479.full.pdf+html>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial Cytochrome C Oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Technology* 3: 294–299. http://www.mbari.org/staff/vrijen/PDFS/Folmer_94MMBB.pdf
- Franco ACNP, Schwarz Jr R, Pierre N, Santos GC (2009) Levantamento, sistematização e análise da legislação aplicada ao defeso da pesca de camarões para as regiões sudeste e sul do Brasil. *Boletim do Instituto de Pesca* 35: 687–699. ftp://ftp.sp.gov.br/ftppesca/35_4_687-699.pdf
- Fransozo A, Costa RC, Castilho AL, Mantelatto FL (2004) Ecological distribution of the shrimp “camarão serrinha” *Artemesia longinaris* (Decapoda, Penaeidea) in Fortaleza Bay, Ubatuba, Brazil, in relation to abiotic factors. *Revista de Investigación y Desarrollo Pesquero* 16: 43–50. <http://www.oceandocs.org/handle/1834/1537>
- Frézal LE, Leblois R (2008) Four years of DNA barcoding: current advances and prospects. *Infection, Genetics and Evolution* 8: 727–736. doi:10.1016/j.meegid.2008.05.005
- Gao TX, Li J, Wang Q, Liu J (2003) Partial sequence analysis of mitochondrial CO1 gene of the Chinese shrimp, *Fenneropenaeus chinensis*. *Journal of Ocean University of Qingdao* 2: 167–170.
- Gopurenko D, Hughes JM (2002) Regional patterns of genetic structure among Australian populations of the mud crab, *Scylla serrata* (Crustacea: Decapoda): evidence from mitochondrial DNA. *Marine and Freshwater Research* 53: 849–857. doi: 10.1071/MF01225
- Guerrero RA, Acha EM, Framin MB, Lasta C (1997) Physical oceanography of the Rio de la Plata Estuary, Argentina. *Continental Shelf Research* 17: 727–742. doi: 10.1016/S0278-4343(96)00061-1
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98. <http://www.mbio.ncsu.edu/JWB/papers/1999Hall1.pdf>

- Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London* 270: 313–321. doi: 10.1098/rspb.2002.2218
- Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astrapes fulgerator*. *Proceedings of the National Academy of Sciences of the USA* 101: 14812–14817. doi: 10.1073/pnas.0406166101
- Hillis DM, Mable BK, Moritz C (1996) *Molecular Systematics*. Sinauer Associates, Sunderland, 655 pp.
- Keskin E, Atar HH (2013) DNA barcoding commercially important aquatic invertebrates of Turkey. *Mitochondrial DNA* 24: 440–450. doi: 10.3109/19401736.2012.762576
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120. doi: 10.1007/BF01731581, <http://www.ncbi.nlm.nih.gov/pubmed/7463489>
- King M (1995) *Fisheries Biology Assessment and Management*. Black Well Science, Oxford, 341 pp.
- Krebs CJ (1994) *Ecology: The Experimental Analysis of Distribution and Abundance*. Harper Collins, New York, 801 pp.
- Laurenzano C, Farias NE, Schubart CD (2012) Mitochondrial genetic structure of two populations of *Uca uruguayensis* fails to reveal an impact of the Rio de la Plata on gene flow. *Nauplius* 20: 15–25. doi: 10.1590/S0104-64972012000100003
- Laurenzano C, Mantelatto FL, Schubart CD (2013) South American homogeneity versus Caribbean heterogeneity: population genetic structure of the western Atlantic fiddler crab *Uca rapax* (Brachyura, Ocypodidae). *Journal of Experimental Marine Biology and Ecology* 449: 22–27. doi: 10.1016/j.jembe.2013.08.007
- Mantelatto FL, Robles R, Schubart CD, Felder DL (2009) Molecular phylogeny of the genus *Cronius* Stimpson, 1860, with reassignment of *C. tumidulus* and several American species of *Portunus* to the genus *Achelous* De Haan, 1833 (Brachyura: Portunidae). In: Martin JW, Crandall KA, Felder DL (Eds) *Crustacean Issues: Decapod Crustacean Phylogenetics*. Taylor and Francis/CRC Press, Boca Raton, 567–579. doi: 10.1201/9781420092592-c29
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*, New Orleans (USA), November 2010, 1–8. doi: 10.1109/GCE.2010.5676129
- Miner BG, Sultan SE, Morgan SG, Padilla DK, Relyeara RA (2005) Ecological consequences of phenotypic plasticity. *Trends in Ecology and Evolution* 20: 685–692. doi: 10.1016/j.tree.2005.08.002
- Nascimento PAM (1983) Observações preliminares sobre a binômia do camarão *Artemesia longinaris* Bate, 1888 (Decapoda, Penaeidea) no Atlântico Ocidental (lat. 29°S–35°S). *Naturalia* 8: 33–47.
- Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications* 13: 146–158. doi: 10.1890/1051-0761(2003)013[0146:PG-DCAT]2.0.CO;2, <http://palumbi.stanford.edu/manuscripts/Palumbi%202003b.pdf>

- Petriella AM, Bridi RJ (1992) Variaciones estacionales del ciclo de muda y la maduración ovárica del camarón (*Artemesia longinaris*). Frente Marítimo 11: 85–92. <http://ctmfm.org/revistas/?i=11#88>
- Pileggi LG, Mantelatto FL (2010) Molecular phylogeny of the freshwater prawn genus *Macrobrachium* (Decapoda, Palaemonidae), with emphasis on the relationships among selected American species. Invertebrate Systematics 24: 194–208. doi: 10.1071/IS09043
- Piola AR, Matano RP, Palma ED, Möller Jr OO, Campos EJD (2005) The influence of the Plata River discharge on western South Atlantic shelf. Geophysical Research Letters 32: L01603. doi: 10.1029/2004GL021638
- Pulliam HR (1988) Sources, sinks and population regulation. American Naturalist 132: 652–661. doi: 10.1086/284880, <http://www.jstor.org/stable/2461927>
- Quan J, Zhuang Z, Deng J, Dai J, Zhang Y (2004) Phylogenetic relationships of 12 Penaeoidea shrimp species deduced from mitochondrial DNA sequences. Biochemical Genetics 42: 331–345. doi: 10.1023/B:BIGI.0000039808.12069.ed
- Rossi N, Mantelatto FL (2013) Molecular analysis of the freshwater prawn *Macrobrachium olfersii* (Decapoda, Palaemonidae) supports the existence of a single species throughout its distribution. PLoS ONE 8: e54698. doi: 10.1371/journal.pone.0054698
- Roughgarden J, May RM, Levin SA (1989) Perspectives in Ecological Theory. Princeton University Press, Princeton, 408 pp.
- Rozas J, Rozas R (1999) DnaSP version 3.0: an integrated program for molecular population genetic and molecular evolution analysis. Bioinformatics 15: 174–175. doi: 10.1093/bioinformatics/15.2.174
- Ruello NV (1975) Geographical distribution, growth and breeding migration of the eastern king prawn, *Penaeus plebejus* Hess. Australian Journal of Marine and Freshwater Research 26: 343–354. doi: 10.1071/MF9750343
- Ruffino ML, Castello JP (1992) Dinámica poblacional del camarón (*Artemesia longinaris*) del sur de Brasil. Frente Marítimo 12: 71–81. <http://ctmfm.org/revistas/?i=12#71>
- Sancinetti GS (2011) Distribuição espaço-temporal e estrutura populacional do camarão *Artemesia longinaris* Bate, 1888 (Crustacea: Decapoda: Penaeidae) no Litoral de Macaé, RJ. Master Thesis. Instituto de Biociências, Universidade Estadual Paulista, Julio de Mesquita Filho, Botucatu, Brazil.
- Schubart CD, Huber MGJ (2006) Genetic comparisons of german populations of the stone crayfish, *Austropotamobius torrentium* (Crustacea: Astacidae). Bulletin Français de la Pêche et de la Pisciculture 380: 1019–1028. doi: 10.1051/kmae:2006008
- Semensato XEG, Di Benedetto APM (2008) Population dynamic and reproduction of *Artemesia longinaris* (Decapoda, Penaeidae) in Rio de Janeiro State, Southeastern Brazil. Boletim do Instituto de Pesca 34: 89–98.
- Sotka EE (2012) Natural selection, larval dispersal, and the geography of phenotype in the sea. Integrative and Comparative Biology 52:538–545. doi: 10.1093/icb/ics084
- Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. doi: 10.1093/bioinformatics/btl446
- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57: 758–771. doi: 10.1080/10635150802429642

- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739. doi: 10.1093/molbev/msr121
- Templeton AR (2006) *Population Genetics and Microevolutionary Theory*. John Wiley & Sons, Hoboken, 705 pp. doi: 10.1002/0470047356
- Terossi M, Mantelatto FL (2012) Morphological and genetic variability in *Hippolyte obliquimanus* Dana, 1852 (Decapoda, Caridea, Hippolytidae) from Brazil and the Caribbean Sea. *Crustaceana* 85: 685–712. doi: 10.1163/156854012X643762
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTALW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680. doi: /10.1093/nar/22.22.4673 <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC308517>
- Vogt G, Huber M, Thiemann M, van den Boogaart G, Schmitz OJ, Schubart CD (2008) Production of different phenotypes from the same genotype in the same environment by developmental variation. *Journal of Experimental Biology* 211:510–523. doi: 10.1242/jeb.008755
- Ward RD (2009) DNA barcode divergence among species and genera of birds and fishes. *Molecular Ecology Resources* 9: 1077–1085. doi: 10.1111/j.1755-0998.2009.02541.x
- Waugh J (2007) DNA barcoding in animal species: progress, potential and pitfalls. *BioEssays* 29: 188–197. doi: 10.1002/bies.20529
- Weber LI, Conceição MB, Ruffino ML, Levy JA (1993) Population genetics of the shrimp *Artemesia longinaris* (Crustacea, Penaeidae) on the south-west Atlantic coast. *Comparative Biochemical Physiology* 106: 1015–1020.
- Wieman AC, Berendzen PB, Hampton KR, Jang J, Hopkins MJ, Jurgenson J, McNamara JC, Thurman CL (2013) A panmictic fiddler crab from the coast of Brazil? Impact of divergent ocean currents and larval dispersal potential on genetic and morphological variation in *Uca maracoani*. *Marine Biology* 161: 173–185. doi: 10.1007/s00227-013-2327-0
- Winkelmann I, Campos PF, Strugnelli J, Cherel Y, Smith PJ, Kubodera T, Allcock L, Kampmann ML, Schroeder H, Guerra A, Norman M, Finn J, Ingao D, Clarke M, Gilbert MTP (2013) Mitochondrial genome diversity and population structure of the giant squid *Architeuthis*: genetics sheds new light on one of the most enigmatic marine species. *Proceedings of the Royal Society B* 280: 20130273. doi: 10.1098/rspb.2013.0273